CLAIMS

1. A bacterial host cell comprising at least two copies of an amplification unit in its genome, said amplification unit comprising:

i) at least one copy of a gene of interest, and

ii) an expressible conditionally essential gene, wherein the conditionally essential gene is either promoterless or transcribed from a heterologous promoter having an activity substantially lower than the endogenous promoter of said conditionally essential gene, and

wherein the conditionally essential gene if not functional would render the cell auxotrophic for at least one specific substance or unable to utilize one or more specific sole carbon source.

2. The cell of claim 1, wherein the bacterial cell is a prokaryotic cell.

3. The cell of claim 2, wherein the bacterial prokaryotic cell is a Gram-positive cell.

4. The cell of claim 3, wherein the bacterial Gram positive cell is a species of the genus *Bacillus*, preferably selected from the group consisting of *Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus coagulans, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus stearothermophilus, Bacillus subtilis, and Bacillus thuringiensis.*

5. The cell of any of claims 1-4, wherein the gene of interest encodes an enzyme, preferably an amylolytic enzyme, a lipolytic enzyme, a proteolytic enzyme, a cellulytic enzyme, an oxidoreductase or a plant cell-wall degrading enzyme, and more preferably an enzyme with an activity selected from the group consisting of aminopeptidase, amylase, amyloglucosidase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, phytase, phenoloxidase, polyphenoloxidase, protease, ribonuclease, transferase, transglutaminase, or xylanase.

6. The cell of any of claims 1–4, wherein the gene of interest encodes an antimicrobial peptide, preferably an anti-fungal peptide or an anti-bacterial peptide.

7. The cell of any of claims 1–4, wherein the gene of interest encodes a peptide with biological activity in the human body, preferably a pharmaceutically active peptide, more preferably insulin/pro-insulin/pre-pro-insulin or variants thereof, growth hormone or variants thereof, or blood clotting factor VII or VIII or variants thereof.

8. The cell of any of claims 1-7, wherein the conditionally essential gene encodes an enzyme from the biosynthetic pathway of an amino acid.

9. The cell of claim 8, wherein the conditionally essential gene encodes one or more polypeptide(s) involved in lysine, leucine or methionine synthesis, preferably the conditionally essential gene is homologous to the *lysA, leuB, metC,* or the *metE* gene from *Bacillus subtilis*, and more preferably the conditionally essential gene is the *lysA, leuB, metC,* or *metE* gene from *Bacillus licheniformis.*

10. The cell of claim 8, wherein the conditionally essential gene is at least 75% identical, preferably 85% identical, more preferably 95% and most preferably at least 97% identical to the *lysA* sequence of *Bacillus licheniformis* shown in SEQ ID NO:48 of WO 02/00907 A1, the *leuB* sequence of *Bacillus licheniformis*, the *metC* sequence of *Bacillus licheniformis* shown in SEQ ID NO:42 of WO 02/00907 A1, or the *metE* sequence of *Bacillus subtilis* shown in positions 997 to 2199 of SEQ ID NO:16.

11. The cell of any of claims 1-7, wherein the conditionally essential gene encodes a glutamyl-tRNA reductase, preferably the conditionally essential gene is homologous to the *hemA* gene from *Bacillus subtilis*, and more preferably the conditionally essential gene is the *hemA* gene from *Bacillus licheniformis.*

12. The cell of any of claims 1-7, wherein the conditionally essential gene is at least 75% identical, preferably 85% identical, more preferably 95% and most preferably at least 97% identical to the *hemA* sequence of *Bacillus licheniformis.*

13. The cell of any of claims 1-7, wherein the conditionally essential gene encodes an enzyme required for xylose utilization, preferably the conditionally essential gene is homologous to the *xylA* gene from *Bacillus subtilis*, and more preferably the conditionally essential gene is homologous to a gene of the xylose isomerase operon of *Bacillus licheniformis*, most preferably to the *xylA* gene of *Bacillus licheniformis.*

14. The cell of any of claims 1-7, wherein the conditionally essential gene encodes a xylose isomerase and is at least 75% identical, preferably 85% identical, more preferably 95% and most preferably at least 97% identical to the *xylA* gene of *Bacillus licheniformis*.

15. The cell of any of claims 1-7, wherein the conditionally essential gene encodes an enzyme required for gluconate utilization, preferably the conditionally essential gene encodes a gluconate kinase (EC 2.7.1.12) or a gluconate permease, more preferably the gene is homologous to the *gntK* gene or the *gntP* gene from *Bacillus subtilis*, and most preferably the gene is the *gntK* or *gntP* gene from *Bacillus licheniformis*.

16. The cell of any of claims 1-7, wherein the conditionally essential gene encodes a gluconate kinase (EC 2.7.1.12) or a gluconate permease or both and is at least 75% identical, preferably 85% identical, more preferably 95% and most preferably at least 97% identical to any of the *gntK* and *gntP* sequences of *Bacillus licheniformis*.

17. The cell of any of claims 1-7, wherein the conditionally essential gene encodes an enzyme required for glycerol utilization, preferably the conditionally essential gene encodes a glycerol uptake facilitator (permease), a glycerol kinase, or a glycerol dehydrogenase, more preferably the conditionally essential gene is homologous to the *glpP, glpF, glpK,* or the *glpD* gene from *Bacillus subtilis*, and most preferably the conditionally essential gene comprises one or more of the *glpP, glpF, glpK,* and *glpD* genes from *Bacillus licheniformis* shown in SEQ ID NO:26 of WO 02/00907 A1.

18. The cell of any of claims 1-7, wherein the conditionally essential gene encodes a glycerol uptake facilitator (permease), a glycerol kinase, or a glycerol dehydrogenase, and is at least 75% identical, preferably 85% identical, more preferably 95% and most preferably at least 97% identical to any of the *glpP, glpF, glpK,* and *glpD* sequences of *Bacillus licheniformis* shown in SEQ ID NO:26 of WO 02/00907 A1.

19. The cell of any of claims 1-7, wherein the conditionally essential gene encodes an enzyme required for arabinose utilization, preferably an arabinose isomerase, more preferably the gene is homologous to the *araA* gene from *Bacillus subtilis*, and most preferably the gene is the *araA* gene from *Bacillus licheniformis* shown in SEQ ID NO:38 of WO 02/00907 A1.

20. The cell of any of claims 1-7, wherein the conditionally essential gene encodes an arabinose isomerase, and is at least 75% identical, preferably 85% identical, more

preferably 95% and most preferably at least 97% identical to the *araA* sequence of *Bacillus licheniformis* shown in SEQ ID NO:38 of WO 02/00907 A1.

21. The cell of any of claims 1-20, wherein the amplification unit further comprises an antibiotic selection marker, preferably the selection marker is flanked by resolvase sites or *res*-sites.

22. The cell of any of claims 1-20, wherein the amplification unit further comprises a resolvase site or *res*-site.

23. The cell of any of claims 1-22, wherein the conditionally essential gene comprised in the amplification unit has at least one transcription terminator located upstream of the gene.

24. The cell of any of claims 1-23, wherein the conditionally essential gene is transcribed from a heterologous promoter having an activity level, when compared with the endogenous promoter of the conditionally essential gene, which is reduced with a factor of 2, preferably 5, more preferably 10, even more preferably 50, and most preferably with a factor of 100.

25. The cell of any of claims 1-23, wherein the conditionally essential gene is promoterless.

26. The cell of claim 25, wherein the gene of interest is located upstream of the conditionally essential gene in the amplification unit, and wherein the two genes are co-directionally transcribed.

27. The cell of claim 26, wherein the conditionally essential gene is expressed by read-through transcription from the gene of interest.

28. A method for producing a protein encoded by a gene of interest, comprising

a) culturing a bacterial host cell comprising at least two duplicated copies of an amplification unit in its genome, the amplification unit comprising:

i) at least one copy of the gene of interest, and

ii) an expressible conditionally essential gene, wherein the conditionally essential gene is either promoterless or transcribed from a heterologous promoter having

an activity substantially lower than the endogenous promoter of said conditionally essential gene,

wherein the conditionally essential gene if not functional would render the cell auxotrophic for at least one specific substance or unable to utilize one or more specific sole carbon source; and

b) recovering the protein.

29. A method for producing a bacterial cell comprising two or more amplified chromosomal copies of a gene of interest, the method comprising:

a) providing a bacterial cell comprising at least one copy of an amplification unit, the unit comprising:

i) at least one copy of the gene of interest, and

ii) an expressible functional copy of a conditionally essential gene, which is either promoterless or transcribed from a heterologous promoter having an activity substantially lower than the endogenous promoter of said conditionally essential gene,

wherein the conditionally essential gene if not functional would render the cell auxotrophic for at least one specific substance or unable to utilize one or more specific sole carbon source;

b) cultivating the cell under conditions suitable for growth in a medium deficient of said at least one specific substance and/or with said one or more specific sole carbon source, thereby providing a growth advantage to a cell in which the amplification unit has been duplicated in the chromosome; and

c) selecting a cell wherein the amplification unit has been duplicated in the chromosome, whereby two or more amplified chromosomal copies of the gene of interest were produced.